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14. ABSTRACT
The aims of this project were to establish reagents and techniques for (a) the advanced assessment of pathogen-specific immune responsiveness, and (b) the immunogenetic characterization of California sea lions. Monoclonal antibodies specific for sea lion antibody isotypes were developed and characterized; they can now be used to measure antibody responses to vaccines. Cytokine genes were sequenced and this data can now be used to develop quantitative assays for measuring cytokine-specific immune responses to vaccines; cytokines are inducible soluble messengers of the immune response. The major histocompatability complex (MHC) class I and II genes were cloned and sequenced. Polymoprhism was identified in the functionally important MHC class II DRB genes. This polymorphism is an indirect measure of the immunologic vigor of a population. Geographical differences in MHC class II DRB were identified and probably reflect exposure to different environmental influences including different pathogens. Assuming that the geographically unique MHC's identified in this study are due in part to pathogen pressures, Navy animals may be at varying degrees of risk when deployed into waters with free-ranging sea lion populations (i.e. different sea lion pathogens may be active in different geographical locations).

15. SUBJECT TERMS
marine mammal, immunology, vaccine, California sea lion

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FINAL REPORT

GRANT #: N00014-00-1-0763

PRINCIPAL INVESTIGATOR: Dr. Jeffrey L Stott

INSTITUTION: University of California

GRANT TITLE: Reagents and Techniques for Vaccine Development and Immune Response Assessment in California Sea Lions

AWARD PERIOD: June 01, 2000 - March 14, 2003

OBJECTIVES: The immediate objectives of this proposal were to: a) establish reagents that can be used to assess the efficacy of vaccination in California sea lions (CSL's) via measurement of post-vaccination cellular (cytokine) and humoral (antibody) immunological responses and b) develop technologies for examining the major histocompatibility complex genotype of CSLs.

APPROACH: Monoclonal antibodies specific for CSL antibody isotypes were developed for use in the qualitative and quantitative evaluation of antibody responses following vaccination. CSL cytokine genes were sequenced using degenerate primers. Polymerase chain reaction (PCR) assays were developed for identification of antigen-induced cytokine mRNA production in lymphocytes following vaccination. Major histocompatability complex genes were analyzed by cloning and sequencing the genes from a large number of CSL's.

ACCOMPLISHMENTS: A hybridoma fusion has been completed for the purpose of developing monoclonal antibodies specific for California sea lion immunoglobulin (antibody) isotypes. Seven monoclonal antibodies were characterized with 3 being specific for the IgG, two being specific for the light chain of Ig (bind all antibody isotypes) and two being specific for IgM.

The sequences of two CSL cytokines (IL-10 and IL-12 p40) and 3 dolphin cytokines (IL4, IL-6, IL-10) were amplified and used successfully in quantitative real time RT-PCR assays to measure their expression in phorbol ester-stimulated peripheral blood-derived leukocytes. A bovine system was successfully modified to detect two additional dolphin cytokines (IL-12 and γ-IFN).

Full-length CSL MHC class I and class II genes have been characterized and a novel molecular technique involving a combination of PCR and denaturing gradient gel electrophoresis

(DGGE) has been adapted for rapidly genotyping both CSL class I and CSL class II MHC genes.

Characterization of California sea lion MHC class II genes resulted in identification of three genes with limited polymorphism and questionable function, and a fourth gene, *Zaca-DRB,* that is unique in its high levels of variability, providing evidence of significant effects of pathogen pressures. Moreover, *Zaca-DRB* constitutes a gene family, comprised of at least 8 loci, each exhibiting limited allelic variability, but present in variable configurations among individuals. This unique aspect of sea lion MHC, in addition to the fact that the Navy California sea lions are potentially subject to different pathogen pressures, suggested it would be important to examine the effects of different environmental influences on MHC. Three populations of free-ranging sea lions were studied and genotypic differences identified in MHC between California sea lions inhabiting the Gulf of California and two distinct Pacific Ocean locations. Additionally, genotypic differences were evident among sea lions from closely-located rookeries in the Gulf of California. The differences in MHC genotypes indicated no substantial gene flows between these populations and among nearby rookeries and have implications for potential disease epidemics in this species. An association has been identified between the presence of MHC class II gene loci *Zaca-DRB-A* and cancer, a disease affecting 18 % of sexually mature sea lions necropsied at The Marine Mammal Center in California. The presence of *Zaca-DRB-A* in the genomic repertoire of an individual confers a 3.3 times greater likelihood of getting cancer. Lastly, differential expression of select MHC DRB loci has been identified in both captive and free-ranging California sea lions; these loci-specific perturbations in gene expression may be the result of multiple environmental stressors.

CONCLUSIONS: Monoclonal antibodies were developed that can detect apparently all antibodies (light chain-specific), IgG specifically and IgM specifically. Polymerase chain reactions were developed for quantitative evaluation of cytokine mRNA production, specifically, γ-IFN, IL-4, IL-6, IL-10 and IL-12 p40. Both MHC class I and class II genes were characterized and genotyping capabilities developed.

SIGNIFICANCE: Development of reagents and techniques for quantitative analysis of CSL antibody and cytokine responses will permit evaluation of prior pathogen exposure and potential vaccine efficacy. The immunogenetic analysis carries considerable implications relative to the deployment of Navy sea lions into national and international waters. Assuming that the geographically unique MHC's identified in this study are due in part to pathogen pressures, Navy animals may be at varying degrees of risk when

deployed into waters with free-ranging sea lion populations (i.e. different sea lion pathogens may be active in different geographical locations).

PATENT INFORMATION: None

AWARD INFORMATION: None

PUBLICATIONS & ABSTRACTS:

Publications

Bowen L, BM Aldridge, F Gulland, J Woo, W Van Bonn, R DeLong, JL Stott and ML Johnson. 2003. Molecular characterization of expressed DQA and DQB genes in the California sea lion (*Zalophus Ccalifornianus*). Immunogenetics 54:332-347.

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